

# High prevalence of *Neisseria gonorrhoeae* strains with reduced susceptibility to fluoroquinolones in Japan

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## Abstract

**Objective**—To study the antibiotic susceptibility of *Neisseria gonorrhoeae* strains isolated in Japan and, in particular, to examine the possibility of emerging fluoroquinolone resistance.

**Materials and methods**—Sixty-nine strains of *Neisseria gonorrhoeae* isolated in 1992 were tested for susceptibility to 15 antibiotics including fluoroquinolones and were seroclassified. Twenty-seven strains isolated from 1981 to 1984 were also evaluated as controls.

**Results**—The MIC<sub>90</sub> values of norfloxacin, ofloxacin, and ciprofloxacin against the isolates from 1992 were 2.0, 1.0, and 0.5 µg/ml, respectively. The MIC<sub>90</sub> values of norfloxacin, ofloxacin, and ciprofloxacin against the isolates from 1981–84 (controls) were 0.25, 0.125, and 0.063 µg/ml, respectively. These results indicate that the MIC<sub>90</sub> values of norfloxacin, ofloxacin, and ciprofloxacin against the strains from 1992 were 8-fold higher than those against the strains from 1981–84. However, there were no significant differences in susceptibility to β-lactams, tetracyclines, macrolides, and spectinomycin between the isolates from 1992 and those from 1981–84. The majority of the isolates belonged to the WII/WIII serogroup. There was no relationship between fluoroquinolone resistance and serogroup.

**Conclusions**—Fluoroquinolones have been used frequently as first-line therapy and have provided excellent clinical efficacy for gonococcal infections for the last several years in Japan. However, our data indicate that a rapid decrease in the susceptibility of *Neisseria gonorrhoeae* to fluoroquinolones is occurring in our country.

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## Introduction

Fluoroquinolones such as ciprofloxacin, norfloxacin, and ofloxacin have excellent in vitro antimicrobial activity against *Neisseria gonorrhoeae*, including penicillin- and tetracycline-resistant strains, and are highly effective as oral single-dose treatment for gonococcal infections.<sup>1–3</sup> Treatment of gonorrhoea with ciprofloxacin is recommended by the World Health Organization (WHO).<sup>4</sup> In Japan, fluoroquinolones have been frequently used as

first-line therapy for gonorrhoea for several years.

However, reduced sensitivity to ciprofloxacin<sup>5,6</sup> and treatment failures with this agent have recently been reported.<sup>5</sup> Development of fluoroquinolone resistance in *Neisseria gonorrhoeae* may be a new worldwide problem complicating the treatment of gonococcal infections.

The present study was performed to investigate the antibiotic susceptibility of *Neisseria gonorrhoeae* isolates in Japan, and particularly to examine the possibility of emerging fluoroquinolone resistance.

## Materials and methods

### Bacterial strains

A total of 82 strains of *Neisseria gonorrhoeae* obtained from men with urethritis attending 15 sexually transmitted disease clinics between February and July 1992 were included in this study. Clinical and epidemiologic information was recorded on the patient's name, age, sexual preference, locality of contact, presence and duration of symptoms, prior antimicrobial exposure, and treatment throughout the study period. Out of the 82 strains, 13 were excluded, that were posttreatment isolates or repeat isolates from the same patient. The remaining 69 strains were tested. Specimens from each patient were inoculated directly onto Thayer-Martin agar (BBL), transported to the laboratory and incubated for 24–48 hours in a 5% CO<sub>2</sub> atmosphere. *Neisseria gonorrhoeae* was identified as Gram-negative diplococci and by oxidase reaction and sugar utilisation patterns. The isolates were stored at –80°C in GDO medium (Nissui) containing gelatin with 10% skim milk, 2% activated charcoal, and 17% glucose until tested.

For comparative purposes, 27 *Neisseria gonorrhoeae* strains (14 β-lactamase-producing and 13 non-β-lactamase-producing) that had been isolated between 1981 and 1984 and stored at –80°C in our laboratory were also evaluated as controls.

### Antibiotic susceptibility testing

The minimum inhibitory concentration (MIC) for all isolates was determined using an agar dilution technique with a GC agar base (Difco) containing 1% Iso VitaleX (BBL) and 2-fold dilutions of antibiotic. Plates were inoculated with 5 µl of 10<sup>6</sup> colony-forming units (cfu)/ml of each isolate using a multipoint inoculator. World Health

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Table 1 MICs of antimicrobial agents against *Neisseria gonorrhoeae* isolated in Japan

Antibiotics	MIC ( $\mu\text{g/ml}$ )					
	1992 (n = 69)			1981-84 (n = 27)		
	50%	90%	Range	50%	90%	Range
Norflaxacin	0.5	2.0	0.004-8.0	0.063	0.25	0.004-2.0
Ofloxacin	0.5	1.0	0.002-2.0	0.063	0.125	0.004-1.0
Ciprofloxacin	0.063	0.5	$\leq 0.001$ -0.5	0.008	0.063	$\leq 0.001$ -0.25
Erythromycin	0.5	2.0	0.063-2.0	0.5	1.0	0.063-2.0
Azithromycin	0.125	0.25	0.016-0.5	0.125	0.25	0.031-0.5
Tetracycline	1.0	2.0	0.125-4.0	1.0	4.0	0.25-4.0
Doxycycline	1.0	1.0	0.125-4.0	1.0	2.0	0.125-4.0
Spectinomycin	16.0	16.0	4.0-32.0	16.0	16.0	8.0-32.0
Cefotaxime	0.031	0.125	0.004-0.25	0.031	0.125	0.004-0.25
Ceftriaxone	0.016	0.063	0.004-0.063	0.016	0.063	0.002-0.063
Cefixime	0.016	0.031	0.002-0.063	0.016	0.063	0.004-0.063
Aztreonam	0.125	0.5	0.031-0.5	0.125	0.25	0.031-0.5
Imipenem	0.063	0.125	0.008-0.5	0.063	0.125	0.008-0.5
Penicillin G*	0.25	1.0	0.016-2.0	1.0	2.0	0.063-2.0
Piperacillin*	0.063	0.125	$\leq 0.008$ -0.25	0.125	0.25	$\leq 0.008$ -0.5

\*non-PPNG strains only: 1992;n = 67, 1981-84;n = 13

Organization *Neisseria gonorrhoeae* reference strains A-E (kindly supplied by Dr. J. W. Tapsall, The Prince of Wales Hospital, Australia) were included as quality standards. The plates were incubated for 24 hours at 35°C in a 5% CO<sub>2</sub> atmosphere. MICs were read as the lowest concentration of antibiotic that inhibited growth.

The tested antibiotics were norflaxacin, ofloxacin, ciprofloxacin, erythromycin, azithromycin, tetracycline, doxycycline, spectinomycin, cefotaxime, ceftriaxone, cefixime, aztreonam, imipenem, penicillin G, and piperacillin. All of the antibiotics were obtained as powders of stated potency from their manufacturers.  $\beta$ -lactamase production was tested by an acidometric assay (Oxoid).

#### Serological classification

Classification into serogroups (WI and WII/WIII) was performed by coagglutination using the Phadebact Monoclonal GC Test™ (Pharmacia).

#### Statistical analysis

The proportions of isolates from 1992 with reduced sensitivity to norflaxacin, ofloxacin, and ciprofloxacin were compared with those of isolates from 1981-84 using the  $\chi^2$  test. There are no established categories that define reduced sensitivity to these antibiotics.

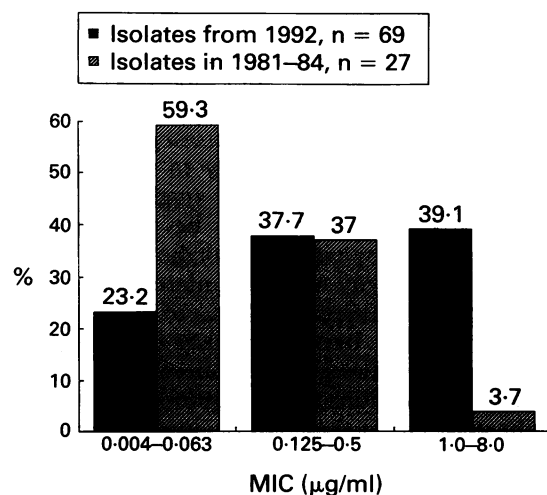


Figure 1 Distribution of norflaxacin susceptibility.

Therefore, we chose three categories of sensitivity as follows:  $\leq 0.063$ , 0.125-0.5, and  $\geq 1.0$   $\mu\text{g/ml}$  for norflaxacin;  $\leq 0.016$ , 0.031-0.25, and  $\geq 0.5$   $\mu\text{g/ml}$  for ofloxacin;  $\leq 0.008$ , 0.016-0.063, and  $\geq 0.125$  for ciprofloxacin. The MICs in the third category indicate reduced sensitivity.

## Results

### Antibiotic susceptibility

The MIC<sub>50</sub>, MIC<sub>90</sub>, and MIC ranges of the 15 tested antimicrobial agents against the 69 isolates from 1992 and the 27 isolates from 1981-84 are shown in table 1. Only 2 (2.9%) of the 69 strains from 1992 were  $\beta$ -lactamase-producing *Neisseria gonorrhoeae* (PPNG).

Therefore, for the studied penicillins the results of the non-PPNG strains were compared. The MIC<sub>90</sub> value of norflaxacin against the isolates from 1992 was 2.0  $\mu\text{g/ml}$ , while that against the isolates from 1981-84 was 0.25  $\mu\text{g/ml}$ . Therefore, the MIC<sub>90</sub> value of norflaxacin against the isolates from 1992 was 8-fold higher than that against isolates from 1981-84. The MIC<sub>90</sub> value of ofloxacin against the strains from 1992 (1.0  $\mu\text{g/ml}$ ) was also 8-fold higher than that against the strains from 1981-84 (0.125  $\mu\text{g/ml}$ ). Furthermore, the MIC<sub>90</sub> value of ciprofloxacin against the strains from 1992 (0.5  $\mu\text{g/ml}$ ) was 8-fold higher than that against the strains from 1981-84 (0.063  $\mu\text{g/ml}$ ).

In general, the MIC<sub>90</sub> values of the other tested antibiotics against the isolates from 1992 were equal, 2-fold higher, or 2-fold lower than those against the isolates from 1981-84.

The incidence of strains from 1992 with reduced sensitivity to norflaxacin (39.1%) was significantly higher than that of strains from 1981-84 (3.7%;  $p < 0.001$  fig 1). There was also a significant difference in the incidence of reduced sensitivity to ofloxacin between the strains from 1992 (40.6%) and those from 1981-84 (3.7%;  $p < 0.001$ , fig 2). Furthermore, a significantly higher incidence of reduced sensitivity to ciprofloxacin was observed in the strains from 1992 (47.8%), compared with those from 1981-84 (3.7%;  $p < 0.001$ , fig 3).

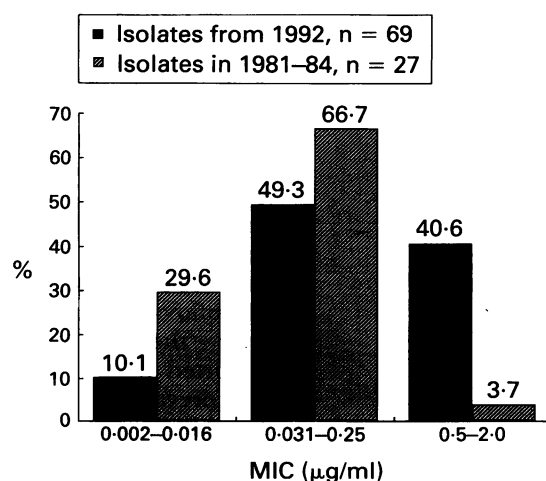


Figure 2 Distribution of ofloxacin susceptibility.

Figure 3 Distribution of ciprofloxacin susceptibility.

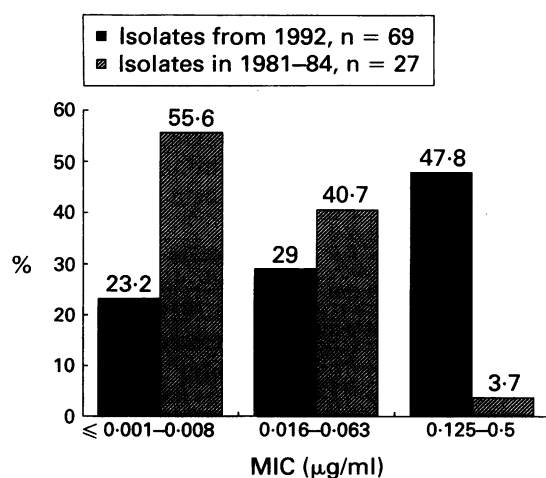


Table 2 Serogroups of *Neisseria gonorrhoeae* strains isolated in Japan

Serogroups	No. of strains (%)	
	1992	1981-84
WI	2 (2.9)	0
WII/WIII	66 (95.7)	27 (100)
WI and WII/WIII	1 (1.4)	0
Total	69 (100)	27 (100)

#### Serological classification

Of the 69 isolates from 1992, 66 (95.7%) belonged to the WII/WIII serogroup, 2 (2.9%) belonged to the WI serogroup, and 1 (1.4%) reacted with both WI and WII/WIII, while all 27 (100%) isolates from 1981-84 belonged to the WII/WIII serogroup (table 2). Most of the isolates were in the WII/WIII serogroup. Therefore, there was no relationship between fluoroquinolone resistance and serogroup.

#### Discussion

Gonococcal resistance to antimicrobial agents is an increasing problem in the treatment of gonorrhoea. A high prevalence of plasmid-mediated high-level or chromosomally mediated low-level resistance to penicillin or tetracycline has been reported in southeast Asia<sup>7-10</sup> and in African countries.<sup>11-13</sup>

Oral single-dose treatment with a fluoroquinolone is now indicated as first-line therapy for gonorrhoea in countries with the problem of antibiotic multiresistance because fluoroquinolones have strong antimicrobial activity against *Neisseria gonorrhoeae*, including strains resistant to penicillin and tetracycline. In addition, oral single-dose treatment is very convenient and cheap. However, the appearance of strains showing reduced sensitivity to ciprofloxacin in vitro<sup>5,6</sup> and treatment failure with the antibiotic have recently been reported in the United Kingdom,<sup>5</sup> although the prevalence of strains resistant to fluoroquinolones is not yet a major problem in that country.<sup>14,15</sup>

Our results demonstrated that there was significantly higher prevalence of reduced sensitivity to norfloxacin, ofloxacin, and

ciprofloxacin among isolates from 1992, compared with isolates from 1981-84. The MIC<sub>90</sub> values of norfloxacin, ofloxacin, and ciprofloxacin against the strains from 1992 were 2.0, 1.0, and 0.5 µg/ml, respectively, while those against the strains from 1981-84 were 0.25, 0.125, and 0.063 µg/ml, respectively. A decrease in susceptibility of gonococcal isolates to fluoroquinolones has also been observed in the Philippines. Clendennen *et al.*<sup>7</sup> reported that the MIC<sub>90</sub> values of norfloxacin, ofloxacin, and ciprofloxacin against *Neisseria gonorrhoeae* isolated in 1989 in the Philippines were 2.0, 0.625, and 0.25 µg/ml, respectively. Therefore, the MIC<sub>90</sub> values of fluoroquinolones against *Neisseria gonorrhoeae* in the Philippines are similar to those of isolates from 1992 in our country. Furthermore, in Rwanda<sup>13</sup> decreasing susceptibility of gonococci to norfloxacin and ofloxacin has been observed. Although *Neisseria gonorrhoeae* strains with resistance to fluoroquinolones are not yet widespread in the United Kingdom,<sup>14,15</sup> Australia,<sup>16</sup> New Zealand,<sup>17</sup> Thailand,<sup>8</sup> Hong Kong,<sup>10</sup> Zaire,<sup>11</sup> and the Gambia,<sup>12</sup> the increase in fluoroquinolone-resistant *Neisseria gonorrhoeae* strains may become a major problem in these countries in the future.

The reasons for this outbreak of *Neisseria gonorrhoeae* strains with reduced sensitivity to fluoroquinolones in Japan are not clear. One of the major reasons may be that fluoroquinolones have been increasingly used as empirical first-line treatment for gonorrhoea for the last several years in our country. Another possibility is that a new strain of fluoroquinolone-resistant *Neisseria gonorrhoeae* was introduced into Japan from southeast Asian countries such as the Philippines.

There is little information about the relationship between the in vitro MIC of fluoroquinolones against gonococci and clinical efficacy. Gransden *et al.*<sup>5</sup> reported that clinical failure with ciprofloxacin occurred in a patient with an *Neisseria gonorrhoeae* strain showing an MIC of 0.06 µg/ml treated with a 250-mg single dose of ciprofloxacin. Bogaerts *et al.*<sup>13</sup> reported that failure with norfloxacin occurred more often in patients with strains having MICs ≥ 0.06 µg/ml treated with 800-mg single doses. In the present study, we measured the in vitro antimicrobial activity of fluoroquinolones against *Neisseria gonorrhoeae*, but did not evaluate the clinical efficacy of the agents. Because of the high prevalence of *Neisseria gonorrhoeae* isolates with low susceptibility to fluoroquinolones in Japan, it seems that single-dose treatment with these agents may be problematic as first-line therapy for gonorrhoea in our country. Therefore, the antimicrobial sensitivity of *Neisseria gonorrhoeae* to fluoroquinolones and the therapeutic efficacy of these antibiotics should be closely monitored.

Quinolone resistance in gram-negative bacteria involves alterations in DNA gyrase and the permeability of bacteria membranes.<sup>18</sup> Inactivation of quinolones by enzymatic degradation as a mechanism of bacterial resistance has not been reported, and

plasmid-mediated resistance to quinolones has not been proved. The research on mechanisms of quinolone resistance in gonococci is very sparse. One study showed that reduced uptake in a ciprofloxacin-resistant strain of *Neisseria gonorrhoeae* could be a mechanism of gonococcal resistance to this agent.<sup>19</sup> However, the ciprofloxacin-resistant *Neisseria gonorrhoeae* tested in that study was only one strain. Further investigations of the mechanisms of quinolone resistance in *Neisseria gonorrhoeae* are indicated.

There were no significant differences in susceptibility to  $\beta$ -lactams, tetracyclines, macrolides, and spectinomycin between our isolates from 1992 and those from 1981–84. The incidence of PPNG (2.9%) in this study was lower than the incidence of 10% to 24% previously reported in Japan between 1983 and 1986.<sup>20</sup> The observed low prevalence of PPNG may be associated with an increase in the use of fluoroquinolones for gonococcal infections in our country.

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